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A BACTERIOLOGICAL STUDY OF THE SOIL OF LOGGERHEAD KEY, TORTUGAS, FLORIDA

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Inasmuch as the coarse calcareous sands of the islands off the Florida Coast, and of similar ones, represent very recent geological material, and since they, therefore, offer an opportunity of determining the early bacterial flora which establish themselves there, it was decided to carry out some studies on typical samples. Dr. A. G. Mayer, Director of the Marine Biological Laboratory of the Carnegie Institution, situated on Loggerhead Key, Tortugas, Florida, supplied us with the necessary samples for our study. Three large samples were collected, which answer to the following descriptions, for which we are indebted to Dr. Mayer:

No. 1. In region thickly covered with *Suriana maritima* bushes. About twenty feet north of stone wall built in 1868 and in a place where probably no man has trodden for 30 years or more. This sample is of an average depth of about 7 inches beneath the surface.

No. 2. Sand from the surface to 6 inches in depth from the northern end of Loggerhead Key. The region is barren of vegetation, no plants having ever grown within 200 feet of the place from which sample was taken. It is about 6 feet above high tide level on the crest of the island. Probably no man has walked here for 10 months previously.

No. 3. From an average depth of 15 inches below the surface in a place densely wooded with *Suriana maritima*. Same locality as Sample No. 1.

We thus had a soil and a subsoil sample from a part of the island in which large bushes (*Suriana maritima*) have established themselves as a permanent association. We also had a surface sample of very coarse, white, calcareous sand or grits, on which plants have never grown. It is to be noted, also, that there has been little or no opportunity for the contamination of these samples by the habitation or tread of man. The flora which now characterize the soil or sand material must take their origin either from the sea water, which now surrounds and which at one time probably covered them, or from winds carrying dust from older soils. The samples were collected by Dr. Mayer with the greatest care, large sterile bottles with cotton stoppers having been employed as containers. The cotton stoppers were doubly protected against contamination while in transit.

The studies carried out included counts of bacteria in the various samples, isolation and identification of pure cultures of the important bacteria and

fungi growing on mannite, beef, and synthetic agar, the determination of the soils ammonia and nitrate producing powers, of their nitrogen fixing powers, and of the isolation and study of the nitrogen fixing organisms found. In this preliminary note, we give merely a brief general statement anent our findings and reserve for another paper the more detailed discussion of the results obtained.

Bacterial Counts.—In the surface soil from the wooded part of the island, as many as a million organisms per gram were found when beef agar was used as a medium. Less than half as many organisms developed on synthetic agar and only about $\frac{1}{15}$ as many on mannite agar. In the subsoil from the same spot, there were approximately $\frac{1}{15}$ as many organisms developing on beef agar as on the surface soil and of synthetic agar the corresponding number was about $\frac{1}{10}$ that of the surface soil on beef agar and less than $\frac{1}{4}$ that on synthetic agar. The subsoil developed only one organism per gram on mannite agar.

In the highly calcareous sand free from vegetation, there were only about 8000 to 9000 organisms per gram of material on both beef and synthetic agar and only $\frac{1}{10}$ as many on mannite agar.

Nitrogen Transforming Powers of the Soils.—Both soil and subsoil from the wooded part of the island show powers of producing ammonia from dried blood nitrogen about equal to those of a poor sandy soil. The calcareous sand which, as has been observed above, contains relatively few organisms, possesses, nevertheless, a power of producing ammonia about three-quarters as great as that of the other soil material.

As regards nitrifying power, all the soils seem to be very feeble, if indeed they possess any such power appreciably. The amount of nitrate produced by the subsoil of the wooded part of the island seems to be above the limit of error, but, curiously enough, the surface soil produces no nitrate from sulphate of ammonia. The calcareous sand appears to produce a small quantity of nitrate from sulphate of ammonia, but the amount so formed may be within the limit of error. From the small amount of nitrogen which the soil itself contains, which in no case attains 0.01%, all of the samples seem to be powerless to produce nitrate. Dried blood in the quantities used seems to be no more satisfactory than sulphate of ammonia. These facts make possible some interesting speculation as to the nitrogen nutrition of the plants growing on the island, which we shall discuss in a future paper.

Nitrogen Fixing Powers and Organisms.—Perhaps the most interesting results obtained in these studies were those on the nitrogen fixing powers and organisms of the soil materials in question. All the samples gave characteristic Azotobacter films in mannite solution, the surface soil from the wooded land giving the heaviest film and the characteristic deep black pigment formation usually ascribed to *A. chroococcum.* The subsoil from the wooded land and the calcareous sand produced thin discontinuous films and little or no pigment. All the films, on microscopic examination, showed typical Azoto-

bacter cells. Judging, therefore, from the very small number of organisms which are found in the calcareous sand, Azotobacter, a nitrogen fixing organism, seems to be one of the earliest and one of the most numerous organisms. The nitrogen fixing power of the soils as measured by the ordinary laboratory test in solution cultures, in contrast with their nitrogen transforming powers, seem to be as vigorous as those of excellent soils. It is interesting, moreover, that the calcareous sand fixed about $\frac{5}{8}$ as much nitrogen in the tests mentioned as the surface soil from the wooded land and about $\frac{5}{8}$ as much as the subsoil of the same land.

Space does not permit a consideration here of some of the pure cultures of bacteria and fungi which were isolated from the soil samples studied. Three species of Actinomyces found appear to be new and as yet remain unnamed. Some of the common organisms of all soils were found, including bacteria, *Actinomyces*, and fungi. These will all be described in detail in a forthcoming paper, mention of which has already been made above.

It is a privilege to acknowledge again our obligation to Dr. A. G. Mayer for his kindness in sending the samples and for his interest in the work. We also express thanks to Dr. H. J. Conn and to Dr. S. A. Waksman for assisting in identification of a few cultures of bacteria and of *Actinomyces*, respectively.

AUTONOMOUS RESPONSES OF THE LABIAL PALPS OF ANODONTA[1]

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Although the ciliary responses of the labial palps of pelecypods have been much studied, the muscular movements of these organs have been entirely neglected. If one valve of an Anodonta is cautiously chipped off leaving the subjacent mantle-lobe intact and the animal resting in the opposite valve, the mantle-lobe thus freed may be folded back so as to expose the parts of the animal lying within the mantle chamber. In this way the labial palps in an almost undisturbed condition may be exposed and worked upon.

In such a preparation the external palp is to be seen resting on the internal one and both are quite flat. If, now, the external palp is touched with a blunt pointed instrument, particularly in its mid-dorsal region, the organ quickly buckles in on its dorsal edge close to its attachment to the mantle and soon after begins to curl from its free tip toward its attached base. On stimulating the internal palp, it responds as the external one does. Both palps in responding curl away from their opposed faces. The vigor of their response is apparently proportional to the stimulus. Grains of sand dropped on the outer face of the external palp affect it as a slight mechanical stimulus, which calls